THE PATENTS ACT, 1970

IT IS HEREBY CERTIFIED THAT, the annex is a true copy of Application and provisional specification filed on 05.09.2002 in respect of patent application no. 809/MUM/2002 of Bharat serums & Vaccines Ltd., Road No. 27, Wagle Estate, Thane – 400 604, Maharashtra, India, an Indian company incorporated under the Companies Act 1956.

This certificate is issued under the powers vested in me under Section 147 (1) of the Patents Act, 1970.

Dated this 6 th day of November 2003

M.A. Haafeez.

(M.A. HAAFEEZ)
ASST. CONTROLLER OF PATENTS & DESIGNS

Drug Com/IP-1
Rs.
05-09-02

FORM 1

THE PATENTS ACT, 1970 (39 of 1970)
APPLICATION FOR GRANT OF A PATENT
[See sections 5(2), 7]

ORIGINAL

1. We, **Bharat serums & Vaccines Ltd., Road No. 27, Wagle Estate, Thane – 400 604. Maharashtra, India.**
an **Indian company incorporated under the Companies Act 1956,**

2. hereby declare
   a) that we are in possession of an invention titled **"A process for the manufacture of low toxicity, stable combination of Ifosfamide and Mesna solution for parenteral administration."**

   b) that the **provisional specification** relating to this invention is filed with this application.

   c) that there is no lawful ground of objection to the grant of a patent to us.

3. We further declare that the inventors for the said invention are
   a) **Dr. Daftary Gautam Vinod**
   Bharat Serums & Vaccines Ltd., Road No. 27, Wagle Estate
   Thane – 400 604., Maharashtra, India
   Nationality - Indian

   b) **Mr. Pai Srikanth Annappa**
   Bharat Serums & Vaccines Ltd., Road No. 27, Wagle Estate
   Thane – 400 604., Maharashtra, India
   Nationality - Indian

   c) **Ms. Rivankar Sangeeta Hanurmesh**
   Bharat Serums & Vaccines Ltd., Road No. 27, Wagle Estate
   Thane – 400 604., Maharashtra, India.
   Nationality - Indian

4. We claim the priority from the application filed in convention countries, particulars of which are as follows - **None**

5. We state that the said invention is an improvement in or modification of - **Not Applicable**

6. We state that the application is divided out of our application, the particulars of which are given below and pray that this application be deemed to have been filed on _____ under section 16 of the Act. - **Not Applicable**

7. That we are the assignee or legal representative of the true and first inventors.

809/mum/2002
5/9/2002

8. That our address for service in India is as follows :

Srikanth Pai
Bharat Serums & Vaccines Ltd., Road No. 27, Wagle Estate
Thane – 400 604., Maharashtra, India

9. Following declaration was given by the inventors.

We, the true and first inventors for this invention declare that the applicant herein is our assignee.

a) ..... **Dt.** 4/9/02
DR. DAFTARY GAUTAM VINOD

b) ..... **Dt.** 4/9/02
PAI SRIKANTH ANNAPPA

c) ..... **Dt.** 4/9/02
RIVANKAR SANGEETA HANURMESH

10. That to the best of our knowledge, information and belief the fact and matters stated herein are correct and that there is no lawful ground of objection to the grant of patent to us on this application.

11. Following are the attachments with the application.
a) Provisional specification - 3 copies
b) Fee Rs. 5,000.
c) Statement and undertaking on Form 3.

We request that a patent may be granted to us for the said invention.

Dated this 30th day of August 2002.

For BHARAT SERUMS & VACCINES LTD.

DR. DAFTARY GAUTAM VINOD
Director

To
The Controller of Patents
The Patent Office
Mumbai.

- 5 SEP 2002

FORM 2

THE PATENTS ACT, 1970
PROVISIONAL SPECIFICATION
[See section 10]

ORIGINAL

Title : ***"A process for the manufacture of low toxicity, stable combination of Ifosfamide and Mesna solution for parenteral administration"***

Applicant : ***BHARAT SERUMS & VACCINES LTD.,***
Road No. 27, Wagle Estate,
Thane – 400 604. Maharashtra, India.

an Indian company incorporated under the Companies Act 1956,

The following specification describes the nature of the invention

This invention relates to a process for preparation of a low toxicity, stable aqueous ready-to-use compositions of Ifosfamide comprising Ifosfamide, Mesna, 2-hydroxypropyl-β-cyclodextrin (referred to hereinafter as "HPBCD"). This invention is particularly related to a process for preparation of compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD suitable for parenteral administration in human beings and mammals. This invention is more particularly related to a process for preparation of clear aqueous low toxicity compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD that is stable over a period of time thereby making it suitable for ready clinical use.

Background and prior art :

Two main groups of drugs used in the treatment of malignant disease are Alkalyting agents and the antimetabolites. Ifosfamide is one of the widely used antineoplastic drug belonging to the alkalyting agents group. Ifosfamide is given intravenously either by injection as a solution diluted to less than 4% or by infusion. It is used in the treatment of a variety of solid tumors including those of the cervix, endometrium, lung, ovary, testes and thymus as well as in sarcoma and in the treatment of Burkitts lymphoma.

Ifosfamide is a white crystalline powder having a low melting point of 40°C. The powder is also hygroscopic. Both these characteristics of Ifosfamide make it difficult for sterile filling of the dry powder as both temperature and humidity are required to be accurately controlled. Further, as Ifosfamide powder is filled aseptically into sterile containers, maximum precautions are required to maintain sterility of the product.

Even though Ifosfamide powder is freely soluble in water, the solubility decreases on storage. Ifosfamide has been reported to undergo a reversible chemical rearrangement in aqueous solution, which is sensitive to changes in pH. The ratio of these compounds to one another in biological fluids have a bearing on the toxicity and efficacy of Ifosfamide.

Ifosfamide is toxic to the urinary tract and may involve the kidneys as well as the bladder. Hence Ifosfamide is recommended to be administered in association with Mesna. Mesna is Sodium 2-mercaptoethanesulphonate used for the prophylaxis of urothelial toxicity in patients being treated with antineoplastics Ifosfamide or Cyclophosphamide. In the

kidney Mesna disulfide, the inactive metabolite of Mesna is reduced to free Mesna, which has thiol groups that react with the metabolites of Ifosfamide, and Cyclophosphamide, including acroleil, considered to be responsible for the toxic effects on the bladder. The intravenous dose of Mesna is 20% of the dose of Ifosfamide on a weight for weight basis given on three occasions over 15 to 30 minutes at intervals of 4 hours beginning at the same time as the Ifosfamide Injection.

US 4959215 discloses an invention of lyophilised preparation consisting of Ifosfamide and Mesna, Mannitol being used as an inert diluent. The lyophilizate is stable physically showing no discolouration. The speed of dissolution is also claimed to markedly higher compared to the dry filled Ifosfamide.

US 4952575 discloses an invention in which Oxazaphosphorin is dissolved in very high concentrations up to 100% of ethanol. Even though the degradation has been shown to be minimal for Ifosfamide, use of solvents in such a high concentration leads to other problems such as volatility, handling during manufacturing, miscibility with blood. As such alcohol is pharmacologically active which may also affect the person on administration of alcoholic solution of Ifosfamide.

WO 99/18973 discloses an invention in which Ifosfamide in saline solution. The product has been shown to be stable at refrigerated temperatures. The stability data provided does not show satisfactory stability at elevated temperatures.

US 4879286 discloses an invention in which Cyclophosphamide is formulated in a ready-to-dilute solution. This invention uses organic Polyol as a solvent and also 0 to 50% water. The water may be partly replaced by 10 to 30% of ethanol. The ready-to-dilute solutions have been shown to be stable under refrigerated conditions. However, the stability data at elevated temperatures are not sufficient to prove that the product is stable.

As Mesna is required to be given at different intervals concurrently during administration of Ifosfamide, in one aspect of the invention Ifosfamide and Mesna are combined in the same composition to avoid the inconvenience of administering Mesna separately. In another aspect of the invention Ifosfamide and Mesna are combined with HPBCD to give a stable composition so that the product is readily marketable and is convenient to use

without the step of reconstitution and less handling. Surprisingly, the process of invention in which Ifosfamide, Mesna and HPBCD are combined has produced a composition having low toxicity also.

Our main objective of this invention is thus to develop a process for preparing low toxicity, stable compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD, with or without conventional parenteral additives overcoming all the disadvantages of prior arts and make the composition suitable for parenteral administration in human beings and mammals.

Accordingly, the invention relates to a process for preparation of a low toxicity, stable compositions of Ifosfamide comprising Ifosfamide, Mesna, HPBCD, with or without conventional parenteral additives; the process comprising steps of

i) adding Ifosfamide to aqueous solution of HPBCD containing conventional parenteral additives and stirring to bring intimate contact;

ii) adding Mesna to aqueous solution containing conventional parenteral additives with or without HPBCD;

iii) Mixing both the aqueous solutions of Ifosfamide and Mesna, making up the volume with water;

iv) filtering the composition obtained through 2μ and 0.2μ filter successively;

v) filling aseptically the filtrate obtained at the end of step (v) in sterile containers such as vials, ampoules, plastic containers followed by nitrogen purging and sealing the filled containers.

The Ifosfamide content of the composition of this process of invention is from about 1mg/ml to about 200mg/ml of the composition, preferably from about 10mg/ml to 100mg/ml of the composition, more preferably from about 40mg/ml to about 50mg/ml of the composition.

The ratio of Ifosfamide to Mesna is in the range of 10 : 1 to 1 : 2 on a weight basis.

The content of HPBCD in the composition prepared by the process of invention is between 1% to 60% w/v of the composition, preferably between 5% to 40% w/v of the composition, more preferably between 10% to 20% w/v of the composition.

The conventional parenteral additives, which may be used in the process of this invention, contain commonly used additives such as buffers, isotonic diluents, anticrystallising agents, sequestering agents, antioxidants. These conventional parenteral additives when added in the usual recommended range do not affect the clarity and stability of the composition adversely.

Buffers are selected from a group of pharmaceutically acceptable buffer systems such as Phosphate buffer, Citrate buffer, Glycine buffer containing any of the commonly used compounds or a mixture of compounds such as Citric acid, Sodium citrate, Potassium citrate, Glycine, Phosphoric acid, Sodium phosphate, Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Potassium phosphate, Dipotassium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium hydroxide, Potassium hydroxide, Hydrochloric acid. Preferably the buffer used is a mixture of Sodium dihydrogen phosphate and Disodium hydrogen phosphate.

Examples :

The invention will now be illustrated by way of Examples. The Examples are by way of illustration only and in no way restrict the scope of the invention.

All the raw materials used in this Examples were of parenteral grade. Equipments used were of conventional nature. Entire processing was done in an area with a controlled environment. Nitrogen cover was provided while processing the batch.

Example I :

1.	Ifosfamide	-	10g
2.	Mesna	-	2g
3.	HPBCD	-	40g
4.	Disodium hydrogen phosphate	-	0.1g
5.	Sodium dihydrogen phosphate	-	0.06g
6.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution. The HPBCD solution was divided into two equal parts.

Weighed quantity of Ifosfamide was gradually added under stirring to one part of buffered HPBCD solution and mixed for 3 hours.

Weighed quantity of Mesna was gradually added under stirring to the remaining part of buffered HPBCD solution.

Both the above Ifosfamide and Mesna solution were mixed together. The volume was made up to 200ml with water. The product was filtered through 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon coated rubber bungs and sealed using flip off seals.

The composition obtained in this Example was analysed for Ifosfamide content and Mesna content by High Pressure Liquid Chromatography (HPLC) method and was found to contain 52.92mg/ml of Ifosfamide and 10.2mg/ml of Mesna. The composition had a pH of 6.86.

Example II :

The composition obtained in Example I was subjected to acute toxicity studies in mice. Conventional formulation reconstituted as directed by the manufacturer and after mixing with Mesna was used as a control. Both the drug solutions were suitably diluted with 5% Dextrose Injection and administered intravenously. Ifosfamide in the dose range of 500mg/kg, 700mg/kg and 900mg/kg body weight was administered in three different groups of animals, each group consisting of eight animals.

Animals were kept under observation for 14 days. Animals were observed for mortality at the end of 3 days and 7 days.

It was observed that the LD_{50} dose was higher for composition of Example I in comparison with the Conventional formulation.

Composition of Example I			Conventional formulation		
Dose (mg)	*Mortality (%)*		*Dose (mg)*	*Mortality (%)*	
	3 Days	7 Days		3 Days	7 Days
500	0	0	500	50	75
700	0	50	700	100	100
900	75	100	900	100	100

The above data clearly proves that composition of the invention prepared in Example I is less toxic compared to the Conventional formulation.

Example III :

1.	Ifosfamide	-	10g
2.	Mesna	-	2g
3.	HPBCD	-	20g
4.	Disodium hydrogen phosphate	-	0.1g
5.	Sodium dihydrogen phosphate	-	0.06g
6.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution.

Weighed quantity of Ifosfamide was gradually added under stirring to buffered HPBCD solution and mixed for 3 hours.

After 3 hours, weighed quantity of Mesna was gradually added under stirring to the buffered Ifosfamide solution.

The volume was made up to 200ml with water. The product was filtered through 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon coated rubber bungs and sealed using flip off seals.

Example IV :

1.	Ifosfamide	-	10g
2.	Mesna	-	2g
3.	HPBCD	-	80g
4.	Disodium hydrogen phosphate	-	0.1g
5.	Sodium dihydrogen phosphate	-	0.06g
6.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution. The HPBCD solution was divided into two equal parts.

Weighed quantity of Ifosfamide was gradually added under stirring to one part of buffered HPBCD solution and mixed for 3 hours.

Weighed quantity of Mesna was gradually added under stirring to the remaining part of buffered HPBCD solution.

Both the above Ifosfamide and Mesna solution were mixed together. The volume was made up to 200ml with water. The product was filtered through 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon coated rubber bungs and sealed using flip off seals.

Example V :

1.	Ifosfamide	-	10g
2.	Mesna	-	6g
3.	HPBCD	-	20g

4.	Disodium hydrogen phosphate	-	0.1g
5.	Sodium dihydrogen phosphate	-	0.06g
6.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution. The HPBCD solution was divided into two equal parts.

Weighed quantity of Ifosfamide was gradually added under stirring to one part of buffered HPBCD solution and mixed for 3 hours.

Weighed quantity of Mesna was gradually added under stirring to the remaining part of buffered HPBCD solution.

Both the above Ifosfamide and Mesna solution were mixed together. The volume was made up to 200ml with water. The product was filtered through 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon coated rubber bungs and sealed using flip off seals.

Example VI :

1.	Ifosfamide	-	10g
2.	Mesna	-	16g
3.	HPBCD	-	20g
4.	Disodium hydrogen phosphate	-	0.1g
5.	Sodium dihydrogen phosphate	-	0.06g
6.	Water	-	q.s. to 200ml

Weighed quantities of Disodium hydrogen phosphate and Sodium dihydrogen phosphate were dissolved in 160ml of water. Weighed quantity of HPBCD was added and dissolved slowly under stirring in this buffer solution. The HPBCD solution was divided into two equal parts.

Weighed quantity of Ifosfamide was gradually added under stirring to one part of buffered HPBCD solution and mixed for 3 hours.

Weighed quantity of Mesna was gradually added under stirring to the remaining part of buffered HPBCD solution.

Both the above Ifosfamide and Mesna solution were mixed together. The volume was made up to 200ml with water. The product was filtered through 0.2μ filter and filled aseptically in sterile glass vials. The glass vials were closed under aseptic conditions with sterile Teflon coated rubber bungs and sealed using flip off seals.